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A Microscale Approach for the Quantitative Detection of PCBs and PAHs in Small Tissue Masses

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PURPOSE: This technical note outlines an analytical approach for the detection of PCBs and PAHs in very small amounts (c. 100 mg wet weight) of invertebrate tissue, including a brief synopsis of the methodology and comparison with traditional techniques. The intent is to show the abilities and limitations of these techniques, how they compare to recommended target detection limits, and how they might be used to support Corps investigations into contaminant bioavailability. While the PAH microscale method is fairly comparable to the traditional method in terms of both method detection limits and mean absolute recoveries, the PCB microscale method did not perform as well with respect to recoveries. However, the PCB microscale method was technically viable with comparable detection limits, and acceptable inter-replicate variability. Ultimately, the choice of whether a laboratory might apply these microscale methods is dependent upon the specific data quality objectives for a particular project (U.S. Environmental Protection Agency (USEPA) 1995). For bioaccumulation and toxicity testing protocols, the microscale method would in many cases offer adequate analytical sensitivity, precision, and accuracy.

BACKGROUND: Organic contaminants, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), are ubiquitous in urban river, estuarine, and coastal sediments, and can pose significant risks to human health, the environment and the nation's economy (National Research Council (NRC) 1997). Quantifying ecological risk requires assessment of contaminant bioavailability, which is determined by various physical, chemical, and biological processes that control uptake into exposed organisms (NRC 2003). Bioavailability of PAHs, PCBs, and other organic contaminants is assessed using bioaccumulation tests, which have been developed and standardized for use with a varied battery of sediment biota (USEPA/U.S. Army Corps of Engineers (USACE) 1998).

Bioaccumulation tests typically require well-replicated factorial exposures of small invertebrates, and often result in samples of very small wet weight (c. 50-500 mg) for tissue concentration analyses. However, traditional analytical methods are designed to address trace levels of contaminants in significantly larger sample sizes (as much as 20-25 g of tissue sample, USEPA 1996), while the Environmental Chemistry Branch at ERDC generally requests 3- to 4-g tissue samples for such methods. Clearly, there is a need for validated analytical methods capable of analyzing contaminants in much smaller tissue masses.

The microscale methods discussed herein were developed from standard EPA analytical methods (EPA 1996), compensating for a lower initial tissue mass by additional concentration of the final extract volume. Working with lower sample masses or extract volumes is challenging due to difficulties in monitoring final extract volume, the concomitant concentration of compounds that

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interfere with target analyte chromatography, and above all, by excessive and unquantified loss of analytes in this final concentration stage. This technical note presents microscale methods for PAH and PCB analyses, quantifies losses associated with the final concentration stage, and compares their method detection limits and analyte recoveries with those of standard methods.

THE MICROSCALE METHODS: Figure 1 shows a stepwise comparison of both PAH and PCB micromethods with traditional methods used in this laboratory. Details of the microscale methods for PAH and PCB, including extraction, cleanup, final extract concentration and analyses, are discussed in Appendix 1. The microscale analyses differed from traditional methods by adding a final extract concentration stage, and scaling down the tissue cleanup methods.

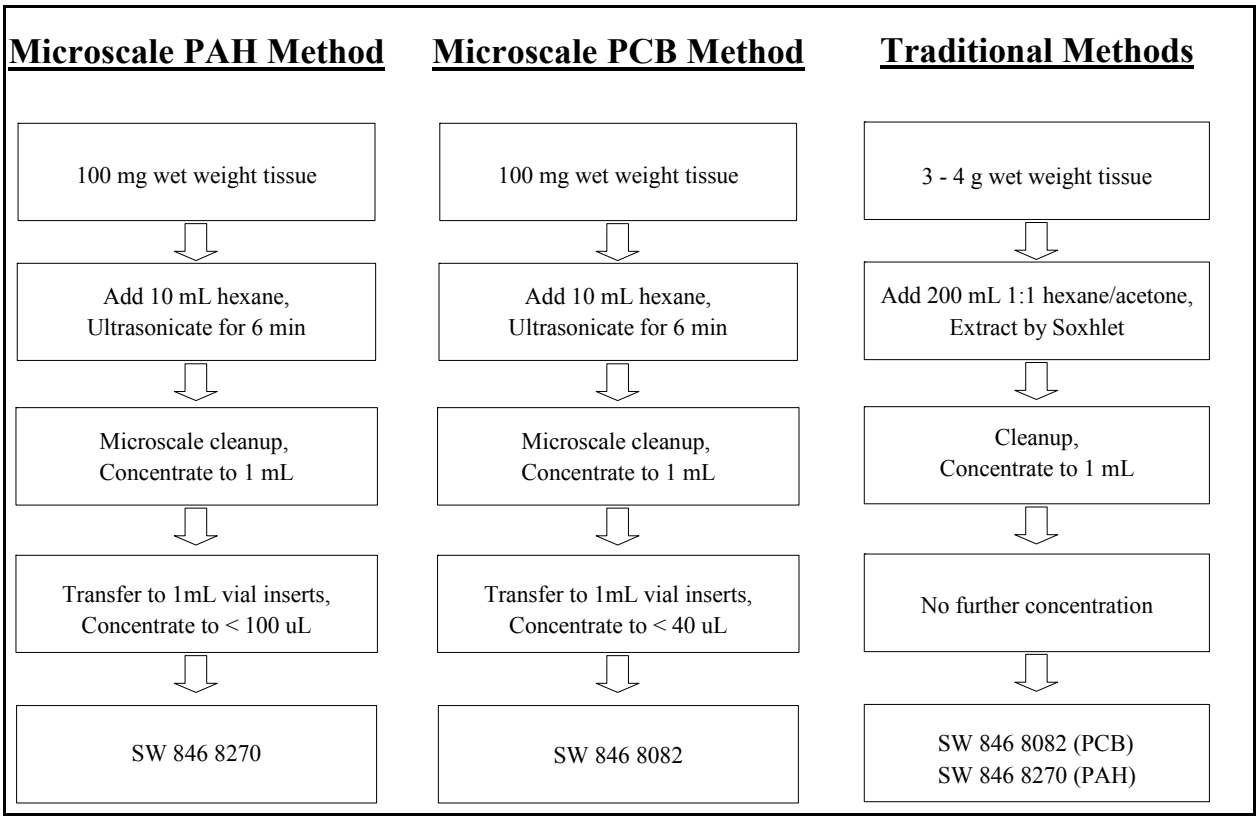

Figure 1. Comparison of microscale PAH, microscale PCB, and traditional methods

Both the PAH and PCB microscale methods compensated for the lower initial tissue mass by concentrating the extract beyond the traditional 1 mL to either 100 µL (PAH microscale method) or 40 µL (PCB microscale method). Internal standards were added prior to this final concentration stage, to aid quantification of analyte concentration. The triglyceride lipid, tricaprin (Sigma, St. Louis, MO) was added to reduce analyte volatilization (i.e. as a 'keeper'), at a final extract concentration of 100 µg tricaprin/mL (PAH microscale method) or 1 µg tricaprin/mL (PCB microscale method).

To support the extract microscale methods, the cleanup stage was scaled down to reflect smaller tissue masses and lower solvent volumes required by these techniques. The traditional cleanup,

eluting tissue extracts through 15 g of silica gel, was replaced with either the PAH microscale cleanup method using 5 g of silica gel, or the PCB microscale cleanup method using 3 g of silica gel. The ability of these scaled-down cleanup methods to remove lipids was tested by loading these smaller columns with a range of lipid masses and measuring the point at which lipid broke through the silica gel into the extract.

Two methods were used to compare the ability of the microscale methods with traditional methods to quantify organic contaminants in either standard reference material (SRM) tissues 2978 or 1974b (National Institute of Science and Technology (NIST) 2000, 2003) or spiked cod filets purchased from a retail store. First, the method detection limits of the two methods were compared, which are defined as the minimum concentration of a target analyte that can be measured, with 99-percent confidence that the concentration is greater than zero. Second, the mean absolute deviations of the methods were compared, which is defined as the average of the absolute differences in percent recoveries from 100 percent for all analytes.

RESULTS AND DISCUSSION:

Lipid Removal by Microscale Cleanup. The lipid breakthrough study showed that both the PAH and PCB microscale cleanup methods were successful in removing lipid from the extract. The lipid breakthrough curves (Figure 2) showed that the microscale PAH cleanup method retained up to 250 mg lipid, and the PCB cleanup method retained up to 100 mg lipid. It can be concluded that both the scaled-down PAH and PCB methods offer effective cleanup for the non-polar lipid fraction in the 100-mg tissue mass intended for the current microscale methods.

PAH Microscale Method Versus Traditional Method. A generalized comparison of PAH method detection limits for the microscale and traditional methods is given in Table 1. Compound-specific results are presented in Appendix 1.

Table 1
Comparison of PAH Microscale Method and Traditional Method

	Method Detection Limit [Mean ± SD]		Mean Absolute Deviation [Mean ± SD]	
Tissue matrix	**Traditional**	**Microscale**	**Traditional**	**Microscale**
Spiked cod tissue	28 ± 23 µg/kg (range = 5–54 µg/kg)	26 ± 21 µg/kg (range = 6–59 µg/kg)	20 ± 22 percent	20 ± 11 percent
SRM 2978	-	-	29 ± 9 percent	8 ± 2 percent

Microscale method detection limits for PAH compounds were 6-59 µg/kg, values not significantly different from those of the traditional method (5-54 µg/kg). The highest microscale method MDL is higher than the target detection limit (TDL) of 20 µg/kg set by USEPA (1995), and lower than the MDL of 200 µg/kg set by the manual for Assessment and Remediation of Contaminated Sediments (Great Lakes National Program Office (GLNPO) 1994). Average percent recoveries for the spiked cod data set were significantly lower ($P = 0.002$) in the microscale method than in the traditional method by approximately 25 percent (data not shown). Mean absolute deviation values were comparable for spiked cod, but not in a standard reference material. Less weight was placed on the comparison using standard reference material, since only three analytes in this material were at concentrations high enough to be detected, compared

to 17 in the spiked cod tissue. The authors suggest that the two approaches are comparable when considering their overall performance across a range of PAHs, but the fact that the microscale method generally recovers at levels significantly lower than the traditional method should not be overlooked.

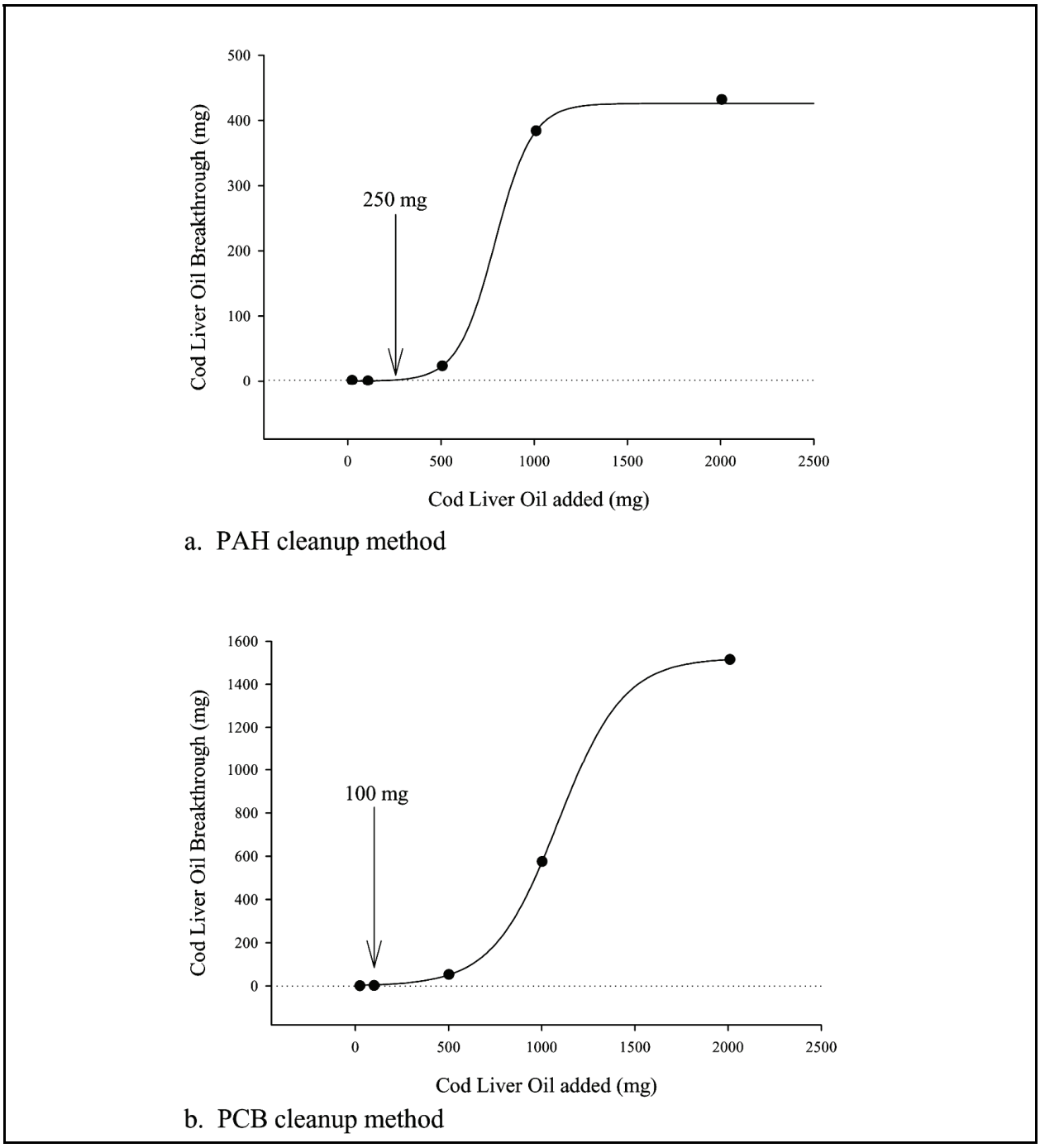

Figure 2. Lipid breakthrough using microscale cleanup silica gel columns

PCB Microscale Method Versus Traditional Method. Table 2 is a generalized comparison of PCB microscale and traditional methods. Compound-specific results are presented in a separate peer-reviewed paper (Jones et al., in publication).

Table 2
Comparison of PCB Microscale Method and Traditional Method

	Method Detection Limits [Mean ± SD (Range)]		Mean Absolute Deviation [Mean ± SD]	
Tissue matrix	Traditional	Microscale	Traditional	Microscale
Spiked cod tissue	1.0 ± 0.3 µg/kg (range = 0.2–1.0 µg/kg)	0.6 ± 0.2 µg/kg (range = 0.5–1.7 µg/kg)	26 ± 17 percent	30 ± 18 percent
SRM 1974b	-	-	22 ± 12 percent	34 ± 13 percent

Method detection limits were higher for the microscale method compared to the traditional method, although the ranges do overlap considerably. The upper MDL for the microscale method is lower than the Target Detection Limit of 2 µg/kg set for PCB congeners by the USEPA (1995), but exceeds the MDL of 0.5 µg/kg set by the manual for Assessment and Remediation of Contaminated Sediments (GLNPO 1994). Average percent recoveries for the spiked cod and standard reference material data sets were significantly lower in the microscale method than in the traditional method by approximately 25 percent for both sets combined (data not shown). Mean absolute deviations for the microscale and traditional methods were not significantly different when using the spiked cod tissue, but were significantly lower for the traditional method than the microscale method using the standard reference material.

CONCLUSION: The choice of whether a laboratory might apply these microscale methods is dependent upon the specific data quality objectives for a particular project (USEPA 1995). The microscale method for PAHs was quite comparable to the traditional method in terms of both method detection limits and mean absolute recoveries. Although the microscale method for PCBs did not perform as well as the traditional method with respect to analyte recoveries, it was demonstrated to be technically viable with comparable detection limits and acceptable inter-replicate variability. It is possible that recoveries might be improved by accepting a larger final extract volume, or by addition of tricaprin to further reduce volatilization.

The microscale method is more labor-intensive than traditional techniques, and does benefit from experienced technicians. This is especially apparent in the final solvent reduction to 40 or 100 µL, which must be monitored carefully to prevent the extract from going to dryness, an outcome that would result in unacceptable losses of target analytes. The small final extract volume limits the number of repeat analyses that can be performed from the same sample.

The microscale approach described in this technical note does offer several advantages. By reducing overall tissue requirements for analytical chemistry, these methods support the current requirements of bioaccumulation and toxicity analyses used routinely by the Corps. In addition, the methods would support future methods involving considerably fewer tissues than those demanded by current EPA methodologies, leading to significant cost savings in animal material and necessitating smaller-scale exposure facilities. Additionally, the microscale method uses less solvent than traditional approaches, reducing the costs associated with solvent purchase and waste disposal. Furthermore, the microscale methods described are based on common standard

methods and techniques, and most commercial labs should be able to implement them without major investment in new equipment. Microscale method development is currently continuing at ERDC, including application to pesticide residue analysis in equally small tissue masses.

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APPENDIX 1. MICROSCALE METHODS FOR PAH AND PCB ANALYSIS IN SMALL TISSUE SAMPLES

Standard Reference Materials, Tissue Homogenates and Chemicals. All analytical chemicals were of high purity or pesticide grade. Standard reference materials (National Institute of Standards and Technology (NIST), Gaithersburg, MD) used in this study were SRM 2978 (NIST 2000), and SRM 1974b (NIST 2003). SRM 1974b (a frozen mussel wet tissue homogenate with moisture content 89.9 percent; lipid content of 0.34 percent) was used to compare microscale methods and traditional methods for PCB congener analysis. The PAH microscale methods and traditional methods were compared using SRM 2978 (a freeze-dried mussel homogenate with moisture content of 7.1 percent, lipid content of 1.8 percent). Method detection limits were calculated using a laboratory-prepared cod fillet homogenate, prepared by processing commercially available cod fillets through a meat grinder five times with thorough mixing between each grinding. The homogenate had a moisture content of 80 percent and a lipid content of 1.4 percent, and was stored at –20 ºC.

Microscale Methods for PAHs. The PAH microscale method is summarized via the flow chart in Figure 1. Aliquots of wet tissue (100 mg) were weighed into certified pre-cleaned 20-mL vials. Surrogates 2-fluorobiphenyl and terphenyl-d14 (Ultra Scientific, North Kingstown, RI) were added to each sample to monitor method efficiency. Hexane (10 mL) was added and each sample extracted twice for a total of 6 minutes using a Fisher Scientific Model 550 Sonic Dismembrator with microtip probe. Combined solvent layers were transferred to a prepared silica gel column.

Cleanup followed a modified version of the procedure described by Warner (1976). Solvent-rinsed chromatography columns (15 x 250 mm, Kimble/Kontes, Vineland, NJ) were packed with a plug of glass wool, followed by 5-g activated silica gel (heated to 130 °C overnight) slurried in hexane, and topped with a small amount of sodium sulfate. Columns were pre-rinsed with dichloromethane followed by hexane. Columns were eluted with 15-mL hexane to remove saturated hydrocarbons, subsequently discarded, followed by 30-mL 20 percent dichloromethane in hexane to elute PAH analytes. Tricaprin (Sigma, St. Louis, MO) was added at a final extract concentration of 100 µg/mL as a 'keeper' to reduce loss of analytes by volatilization, and samples were then concentrated on a Zymark TurboVap II to approximately 1 mL.

For final concentration, extracts were transferred to solvent-rinsed conical-bottom 1-mL vials (Supelco, Bellefonte, PA), and were concentrated under a gentle stream of nitrogen to less than 100 µL. To aid determination of final extract volume, 100 uL of solvent was added to the insert with a syringe and the inserts were marked with a lab marker to indicate the required final volume. After the measuring aliquot was removed, extracts were transferred to inserts using toluene as a rinse. Nitrogen was used to further concentrate each extract if needed. Internal standards (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12) were added at a final concentration of 2 µg/mL and the volume was adjusted to the mark.

PAH Quantitative Analysis. Both microscale and traditional method extracts were analyzed following EPA Method 8270 (U.S. Environmental Protection Agency (USEPA) 1996a) with selected ion monitoring using a Hewlett-Packard 5890 gas chromatograph with HP-5971

detector using 1uL injection volume. Initial temperature was 35 °C (2-min hold time), ramped to 130 °C at 35 °C/minute, a subsequent ramp to 250 °C (1-min hold time) at 12 °C/minute, and a further 10 °C/minute ramp to 300 °C (10-min hold time). Injector, transfer line, and source temperature were 250, 280, and 167 °C, respectively. Helium carrier flow was 0.89 mL/minute at 413 g/cm^2 (5.88 psi) head pressure. In the SIM analysis, for each analyte a primary ion was used for identification and quantitation in addition to a secondary qualifier ion.

Microscale Methods for PCBs. Aliquots of wet tissue (100 mg) were weighed into certified pre-cleaned 20-mL vials. A surrogate, 2,4,5,6-tetrachloro-m-xylene, was added to each sample to monitor method efficiency. Hexane (10 mL) was added and each sample extracted twice for a total of 6 min using a Fisher Scientific Model 550 Sonic Dismembrator with microtip probe. Combined solvent layers were transferred to a prepared silica gel column.

Cleanup followed a scaled-down version of EPA Method 3630C (USEPA 1996a). Solvent-rinsed chromatography columns (15 x 250 mm, Kimble/Kontes, Vineland, NJ) were packed with a plug of glass wool, followed by 3-g deactivated silica gel (3.3 percent moisture) and topped with a small amount of sodium sulfate to dry the extract. Columns were pre-rinsed with 15-mL hexane. Following addition of sample extracts, columns were eluted with 80 mL of hexane. Samples were then concentrated on a Zymark TurboVap II to approximately 1 mL. Extracts were transferred to clear 12-mL vials, 1 mL of concentrated sulfuric acid was added, and the mixture vortexed for 30 s. The hexane layer was transferred to another 12-mL vial and the remaining acid rinsed with a small amount of hexane that was combined with the primary extract. To neutralize and remove traces of acid, each extract was washed with approximately 1 mL of saturated sodium bicarbonate in water. The final extract was concentrated under a nitrogen stream to approximately 1 mL.

Prior to the additional concentration step, extracts were transferred to solvent-rinsed conical-bottom 1-mL vials (Supelco, Bellefonte, PA), and tricaprin (Sigma, St. Louis, MO) was added at a final extract concentration of 1000 ng/mL, as a keeper to reduce loss of analytes by volatilization. Extracts were concentrated under a gentle stream of nitrogen to less than 40 µL. To aid determination of final extract volume, 40 uL of solvent was added to the inserts with a syringe and the inserts were marked at the meniscus with a lab marker. After the measuring aliquot was removed, extracts were transferred to inserts using toluene as a rinse. Nitrogen was used to further concentrate each extract if needed. Internal standards, pentachloronitrobenzene, 4,4'-dibromobiphenyl, and decachlorobiphenyl (Restek, Bellefonte, PA), were added at a final concentration of 50 ng/mL and the volume was adjusted to the mark.

PCB Quantitative Analysis. Both microscale- and traditional-method extracts were analyzed following EPA Method 8082 (USEPA 1996a) using a Hewlett-Packard 5890 series II gas chromatograph equipped with electron capture detectors and dual columns. Agilent (Wilmington, DE) HP-5MS and Supelco (Bellefonte, PA) SPB-octyl columns, both 30 m, 0.25-mm ID, 0.25-µm-film thickness, were used to achieve separation. Initial oven temperature was 130 ºC (2-min hold time), ramped to 255 ºC at 1.4 ºC/minute. The temperature was then raised to 265ºC at a rate of 18º/minute and held for 9 min, resulting in a total run time of 100.8 min. Injector and detector temperatures were 255 and 305 ºC, respectively. The carrier gas mix was helium (1.35 mL/minute) and 5-percent methane in argon (65 mL/minute) gas.

Efficiency Calculations, Method Detection Limits and Statistical Analyses. Microscale methods and traditional methods were evaluated statistically through comparison of method detection limits, and mean absolute deviations.

Method detection limits are defined as the minimum concentration of a target analyte that can be measured and reported with 99-percent confidence that the concentration is greater than zero, and were determined according to standard procedures (USEPA 1996b). Eight replicate samples were spiked into homogenized cod tissue at the low calibration standard concentration (2.0 ng/g for PCB and 0.1 µg/g for PAH). Prior analyses verified that tissues were free of significant background concentrations of PCB and PAH. Samples were extracted and cleanup performed following the above procedures and method detection limits were calculated from the equation:

$$\text{MDL} = t\,\sigma \tag{1}$$

where t is the one-sided t statistic at the 99-percent confidence level for n - 1 degrees of freedom (n = number of replicate analyses) and σ is the standard deviation of replicate concentrations (Tables 1-1 and 1-2).

In addition to percent recoveries, extraction efficiency was evaluated for each method by comparing mean absolute deviations (MAD). Mean absolute deviation uses the average of all analyte absolute deviation, defined as the absolute value of the percent recovery difference from 100 percent:

$$MAD = \frac{\sum^{n} \left| X_i - 100\% \right|}{n} \tag{2}$$

where X_i is the percent recovery of compound i, and n is the number of compounds.

Statistical analysis of data was performed using SigmaStat, version 3.0 (SPSS, Inc., Chicago, IL). Data groups were compared using the pairwise parametric Student's 'T' test, or non-parametric Mann-Whitney rank sum test. A significance level of 0.05 or lower was used for all statistical tests.

Table 1-1
Method Detection Limits for PAH Compounds from Spiked Cod Tissue Matrix, for Both Traditional and Microscale Methods

Compound	Method Detection Limit, µg/kg	
	Traditional	**Microscale**
Naphthalene	19	45
2-Methylnaphthalene	20	31
Acenaphthylene	4.8	6.0
Acenaphthene	6.7	9.8
Fluorene	9.7	13
Phenanthrene	17	11
Anthracene	12	13
Fluoranthene	18	13
Pyrene	27	59
Benz[*a*]anthracene	19	15
Chrysene	20	8.6
Benzo[*b*]fluoranthene	26	19
Benzo[*k*]fluoranthene	24	16
Benzo[*a*]pyrene	23	8.3
Indeno[1,2,3-*cd*)pyrene	19	48
Dibenz[*ah*]anthracene	47	35
Benzo[*ghi*]perylene	54	51
Average	28 ± 23 (range 5–54)	26 ± 21 (range 6–59)

Table 1-2
Method Detection Limits for PCB Congeners from Spiked Cod Tissue Matrix, for Both Traditional and Microscale Methods

IUPAC #	Name	Method Detection Limit, µg/kg	
		Traditional	Microscale
PCB 5	2,3-Dichlorobiphenyl	0.4	0.8
PCB 18	2,2',5-Trichlorobiphenyl	1.0	1.3
PCB 31	2,4',5-Trichlorobiphenyl	0.8	1.1
PCB 44	2,2',3,5'-Tetrachlorobiphenyl	0.7	1.6
PCB 52	2,2',5,5'-Tetrachlorobiphenyl	0.9	1.2
PCB 66	2,3',4,4'-Tetrachlorobiphenyl	0.8	0.9
PCB 87	2,2',3,4,5-Pentachlorobiphenyl	0.8	0.8
PCB 101	2,2',4,5,5'-Pentachlorobiphenyl	0.2	0.8
PCB 110	2,3,3',4',6-Pentachlorobiphenyl	0.8	0.8
PCB 138	2,2',3,4,4',5'-Hexachlorobiphenyl	0.7	1.2
PCB 141	2,2',3,4,5,5'-Hexachlorobiphenyl	0.4	1.7
PCB 151	2,2',3,5,5',6-Hexachlorobiphenyl	0.8	1.1
PCB 153	2,2',4,4',5,5'-Hexachlorobiphenyl	0.4	0.9
PCB 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	0.4	0.7
PCB 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	0.7	1.1
PCB 183	2,2',3,4,4',5',6-Heptachlorobiphenyl	0.7	0.8
PCB 187	2,2',3,4',5,5',6-Heptachlorobiphenyl	0.5	0.9
PCB 206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	0.2	0.5
	Average	1.0 ± 0.3 (range 0.2–1.0)	0.6 ± 0.2 (range 0.5–1.7)

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